Identification of Per- and Polyfluoroalkyl Substances in the Cape Fear River by High Resolution Mass Spectrometry and Nontargeted Screening

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⊛ Supporting Information

ABSTRACT: Ongoing chemical development in response to regulation of historical perfluorinated compounds, (i.e., perfluorooctanoic acid (PFOA) and perfluorooctanesulfonic acid (PFOS)) has resulted in a proliferation of novel per- and polyfluorinated species. Screening and monitoring for these emerging chemicals benefit from a nontargeted approach due to a lack of necessary standards and a paucity of information about the replacement chemistries. In this paper, we apply nontargeted screening to the Cape Fear River of North Carolina, a fluorochemically impacted watershed. The continued presence of perfluorinated ether acids was confirmed, with a total of 37 unique chemical formulas comprising 58 isomers detected. Structural determination was carried out by LC-MS/MS to determine isomeric structures where possible. Novel structures determined included perfluorinated ether acid species containing two acidic sites, polyfluorinated ether acids containing a single hydrogenation, and previously unreported perfluorinated ether acids. Compounds identified by an initial nontargeted screen were monitored over repeated sampling to track long-term reductions in PFAS content during emission source control. Hierarchical clustering of the time course data was used to associate groups of chemicals based on their trends over time. Six clusters were identified and showed some similarity in chemical class; they are believed to represent the byproducts of different fluorochemical production lines.
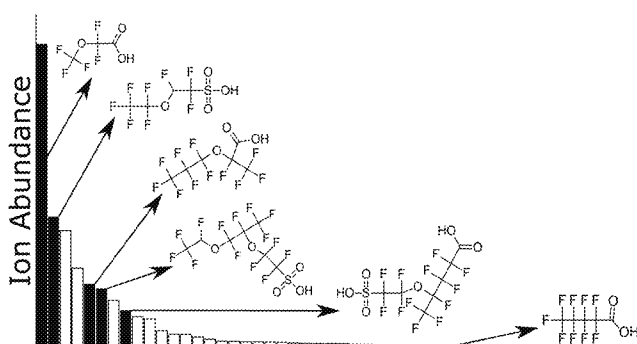
■ INTRODUCTION

Fluorinated polymers and fluorosurfactants are constantly evolving due to in-demand consumer and industrial products with a long and complicated history of environmental impacts.[1] A consequence of the ubiquity of per- and polyfluoroalkyl substances (PFASs) in industry is their extensive environmental infiltration due to their use as manufacturing aids, production as waste, and release as degradations and side-products of manufactured products. PFASs possess exemplary thermochemical inertness, and fluorinated carbons are resistant to biological breakdown,[2] which is the cause of their persistent nature in the environment[3] and was the driving rationale for the erroneous assumption of biological inertness for over 25 years.[4]

The most historically dominant PFAS species, perfluorooctanesulfonate (PFOS) and perfluorooctanoic acid (PFOA), were used extensively before triggering domestic and international action due to concerns about their negative toxicological effects and bioaccumulation potential.[5−7] Long-term toxicological studies demonstrated that these species have broad spectrum influence and require exposure controls.[8,9] Coinciding with the environmental studies in the previous decade, domestic manufacturing began phasing out PFOA and PFOS,[10,11] but the chemical qualities of fluorochemicals are difficult, or impossible, to replicate using nonfluorinated species.[12,13] Consequently, industries developed fluorinated alternatives, including short-chain PFASs and perfluoroalkyl ether acids, to replace legacy long-chain PFASs.[14,15]

A chief difficulty with chemical phase-out is the assurance that replacement chemicals from the same class demonstrate improved environmental and human health profiles. Historically, reliance on chemicals within a single chemical class has not markedly overcome fundamental issues presented by the original compounds (e.g., the replacement of polychlorinated biphenyls with short-chain chlorinated paraffins or the

65 emerging replacements for brominated flame retardants).[16,17]
66 Due to the aforementioned uniqueness of fluorinated
67 compounds, there may be challenges to the prospect of
68 alleviating the toxicological effects associated with PFOA/
69 PFOS or legacy PFASs by using related chemistries.[13,18] One
70 company has introduced a new line of "short-chain" PFOS
71 replacements which exhibit nearly identical structures but
72 potentially better biological clearance rates.[19] Others have
73 introduced branched polymeric surfactants with "low-bio-
74 accumulation potential".[20] Still, more manufacturers have
75 created species with interspersed ether-oxygen linkages[21] or
76 partial hydrogenation.[22] The net result is a proliferation of
77 product chemicals, precursors, and degradation products with
78 limited publicly available toxicological and physical data due to
79 the proprietary nature of these compounds. Monitoring this
80 vast new chemical landscape requires the development of new
81 analytical methodologies.

82 High-resolution mass spectrometry (HRMS) has a proven
83 track record in compound elucidation and environmental
84 screening that is well suited to the task of identifying and
85 monitoring emerging pollutants through accurate chemical
86 prediction and structural elucidation.[23–28] HRMS approaches
87 have been applied to numerous environmental samples
88 collected around fluorochemical point sources to identify
89 new unregulated compounds. For example, new structures
90 were resolved in water located around Chinese manufacturing
91 facilities[28] and in river water from domestic sites.[29] Potential
92 structures were assigned to numerous PFASs in Alabama[26] and
93 previously in the Cape Fear River of North Carolina.[29,30] In
94 each case, spatial and/or temporal sampling was used to
95 compare impacted and baseline water samples, while
96 identifying molecular features associated with the fluorochem-
97 ical waste stream.

98 In North Carolina, discovery of the chemical hexafluor-
99 opropylene oxide dimer acid (HFPO-DA) sparked intense
100 scrutiny of its long-term effects, resulting in the establishment
101 of a preliminary NC state drinking water health goal.[31] Since
102 initial reporting by media outlets,[32] the "chemical" GenX has
103 been used as a public synonym for HFPO-DA. While GenX
104 has occupied the center of the debate regarding fluorochemical
105 contamination in the Cape Fear River, the presence of other
106 fluorinated chemicals of related classes is also of interest. We
107 combine the previously demonstrated nontargeted approach
108 with techniques for structural assignment by tandem mass
109 spectrometry (MS/MS) to identify the additional fluorochem-
110 ical species affecting the Cape Fear watershed.

111 ■ METHODS

112 **Sample Collection.** Surface water grab samples were
113 collected in May 2017 from the main flow of the Cape Fear
114 River and its tributaries at the William O. Huske Lock and
115 Dam. An "upstream" sample was collected approximately 2
116 miles upstream of the dam, representative of the background
117 contents of the river. A "downstream" sample was collected
118 immediately above the dam, representative of the impacts of
119 nearby (<1 mile) manufacturing outfall (Supporting Informa-
120 tion Figure 91). Longitudinal grab samples of the manufactur-
121 ing outfall were collected weekly by the North Carolina
122 Department of Environmental Quality (NCDEQ) throughout
123 the summer of 2017,[33] and follow-up samples were collected at
124 the original sampling locations in February 2018. At each site,
125 water was collected in a precleaned one liter high-density

126 polyethyelene (HDPE) Nalgene bottle according to an
127 established procedure.[34]

128 The collected water was processed as previously described,
129 after stabilization with the addition of 5 mL 35% nitric acid.[26]
130 In brief, one liter of water was measured in an HDPE
131 graduated cylinder, and the sample bottle was washed with 10
132 mL of methanol to desorb analytes adhered to the interior of
133 the bottle. The water and methanol were pooled in the bottle
134 and filtered using a Whatman GF/A glass fiber filter. From this
135 filtered sample, 500 mL of liquid was concentrated using a
136 SPC10-P Sep-Pak concentrator onto an Oasis WAX Plus SPE
137 cartridge (225 mg sorbent, 60 μm particle size) that had been
138 preconditioned with methanol (MeOH) and water per
139 manufacturer instructions. SPE cartridges were washed with
140 4 mL of pH 4 acetate buffer, followed by 4 mL of neutral
141 methanol, and then compounds were eluted in 4 mL of 0.1%
142 NH$_4$OH in MeOH. The methanol wash fraction could have
143 contained nonacidic PFAS, but preliminary experiments did
144 not detect these in samples from the Cape Fear, so they were
145 discarded in the work for this manuscript. The eluent was
146 evaporated under a nitrogen stream at 40 °C to a final volume
147 of 1 mL and diluted 4-fold with 2.5 mM ammonium formate. A
148 method blank was prepared in parallel using laboratory
149 deionized water.

150 **Sample Analysis and Data Processing.** Initial mass
151 spectrometry (MS) analysis on the collected water samples was
152 carried out by liquid chromatography coupled to time-of-
153 flight (TOF) in a fashion previously described;[29] instrument
154 details are located in the Supporting Information. MS sample
155 data was postprocessed using a software workflow of Agilent
156 ProFinder, MassHunter, and Mass Profiler. Samples were
157 subjected to batch recursive molecular feature extraction
158 (MFE) in ProFinder using previously determined thresholds
159 for this instrument and separation gradient (Supporting
160 Information Table 3). A molecular feature, as defined by the
161 software, consists of a specific isotopic distribution with a
162 distinct extracted ion chromatogram peak abundance and is
163 assigned a monoisotopic mass and retention time. Molecular
164 features identified in the method blank were removed from the
165 sample data, and the remaining values were exported for
166 comparison in Mass Profiler. Samples collected downstream of
167 the known fluorochemical point source were compared to
168 upstream samples, which should be free of species introduced
169 by the intervening facility. Pairwise comparison was used to
170 identify features unique to the downstream samples as well as
171 species which were significantly increased (>3-fold increase in
172 integrated peak area). The list of the top 100 most abundant
173 unique and statistically significant species was exported and
174 prepared as an inclusion list for further MS/MS investigation.

175 Structural investigation of the molecular features was
176 performed using an Agilent 1260 Infinity UPLC coupled to
177 an Agilent 6530 quadrupole time-of-flight (QTOF). Parame-
178 ters for LC separation were the same as the initial TOF
179 analysis, but MS settings were adjusted to allow collection of
180 MS/MS fragmentation spectra of the targeted inclusion list
181 from the TOF analysis. Modifications to the MS collection are
182 detailed in the Supporting Information.

183 The raw data collected from QTOF MS/MS experiments
184 was processed using ProFinder, followed by filtering to isolate
185 features for closer inspection. Comparison of precursor and
186 fragment masses allowed a maximum 5 ppm precursor and 20
187 ppm fragment mass error; average mass error was ~1 ppm,
188 consistent with the performance specifications of the instru-

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189 ment. Features were individually examined to determine
190 structures where possible. For each mass-to-charge (m/z)
191 identified at the formula level or better (Confidence 4+), an
192 ±10 ppm extracted ion chromatogram of the theoretical
193 precursor mass was generated to determine retention times
194 (Supporting Information Figures 1−83). Composite MS/MS
195 spectra consisting of multiple averaged MS/MS scans at
196 collision induced dissociation (CID) energies of 10 and 25 V;
197 spectra were generated for individual isomers of each precursor,
198 where possible.

199   The list of molecular features was sorted by integrated peak
200 area and the isotopic distributions for each of the molecular
201 features; a putative empirical formula was assigned using
202 MassHunter's formula generator tool. This assignment is a
203 score obtained by matching the feature's accurate mass and
204 isotopic distribution with theoretical values calculated using
205 known isotopic distributions of a potential formula's
206 constituent elements (i.e., 1.1% ^{13}C, 0.4% ^{15}N, etc.). Allowed
207 atoms included C, H, N, O, S, F, Cl, Br, and P. For compounds
208 with only poorly scoring isotopic matches (e.g., <70%),
209 background subtraction of the precursor spectrum was carried
210 out against a background of the 0.2 min regions immediately
211 preceding and following the chromatographic peak, and a
212 second attempt at formula assignment was made.

213   The MS/MS spectra collected for each molecular feature
214 were manually examined to ascertain the accuracy of the
215 precursor formula and attempt to resolve molecular structures.
216 Emphasis was placed on chemicals demonstrating a negative
217 mass defect—the difference between the exact and nominal
218 mass of the compound, a common signature of halogenation.[22]
219 It is worth noting that this is only a rough proxy for the
220 inclusion of critical atoms of interest (i.e F, Cl, Br) in small
221 molecules and significant supporting data is necessary to
222 classify an unknown as a PFAS.[35] Fragment ions were assigned
223 partial empirical formulas based on exact monoisotopic mass
224 using a similar process to the precursor mass but using the
225 precursor formula as the list of allowed atoms. Potential
226 formulas were compared against the existing EPA's Comptox
227 Chemicals Dashboard,[36] an internally maintained PFAS
228 database, and extant literature, including publications, patents,
229 and the CAS registry, to obtain known potential structures.
230 Potential structures were also generated *de novo* based on
231 observed fragments and related chemicals. A degree-of-
232 confidence assessment for each chemical feature was assigned
233 based on the Schymanski classification.[37] Briefly, where verified
234 standards existed for a proposed structure, comparison with
235 the retention time and MS/MS structure of the standard was
236 carried out; matches were assigned a confidence level of 1. In
237 the absence of a confirmed standard, fragment ions were
238 compared to available standards with similar substructures and
239 empirical or *in silico* library spectra; probable structures were
240 assigned a confidence level of 2. Where structures could not be
241 completely resolved by MS/MS, partial elucidation of chemical
242 class/structure was based on diagnostic fragments and
243 relationship to identified compounds; tentative candidates
244 were assigned a confidence level of 3. In absence of quality
245 MS/MS spectra or where incompatible tentative candidates
246 could not be resolved, only a molecular formula was
247 determined; molecular formula level assignments were given
248 a confidence level of 4. Features with lower confidence were
249 not reported here.

250   **Clustering of Non-Targeted Chemical Features by**
251 **Time Trend.** The samples collected in May 2017 and

252 February 2018 bracketed significant changes to the Cape
253 Fear River as the result of regulatory actions by NCDEQ
254 against the Chemours company.[33] As part of their ongoing
255 monitoring, the state collected water samples proximate to the
256 waste outfall into the Cape Fear River over several months
257 during the summer of 2017. These samples were acquired from
258 NCDEQ, analyzed by TOF-MS as described, and the ion
259 abundances for the species were monitored over the sampling
260 time points. Abundance measurements for each chemical were
261 normalized to the highest abundance for the feature across all
262 sampling points for that chemical. Time trends of chemical
263 features were hierarchical clustered for similarity with *hclust* in
264 R (3.4.4) using Euclidean distance and the "Ward.D2"
265 clustering criterion;[38] cluster p-values were calculated using
266 *pvclust*[39] with 10000 bootstrap iterations and filtered to $p <$
267 0.05.

■ RESULTS AND DISCUSSION 268

Feature extraction from surface water collected upstream of a 269
fluorochemical manufacturing site yielded ~120 distinct 270
molecular features. Features with PFAS-like MS signatures 271
were all from the legacy perfluorinated acids (PFBA, PFPeA, 272
PFHxA, PFHpA, PFHxS, PFOA, and PFOS). No additional 273
PFAS species were detected in the samples. The existence of 274
these fluorinated compounds in the surface water is expected, 275
because of their persistent nature and ubiquitous use in 276
preceding years, and has been previously reported.[40−44] 277

Feature extraction from the sample immediately downstream 278
from the fluorochemical manufacturer yielded a significantly 279
larger amount of ~260 distinct molecular features of which 280
nearly 200 possess the negative mass defect marker indicative 281
of potential halogenation (Supporting Information Table 1). A 282
comparison of the total signal intensity of species with a 283
negative mass defect in the upstream and downstream samples 284
indicates that while ~15% of the total ion current for the 285
upstream sample is resultant from perfluorinated acids above, 286
the downstream sample is, by total ion intensity, >90% 287
composed of species with a negative mass defect. Further, the 288
ambient PFASs detected upstream make up <1% of the total 289
ion intensity in the downstream sample. Clearly, wastewater 290
discharge from the fluorochemical manufacture introduces a 291
significant number of compounds to the river besides the 292
legacy perfluorinated acids detailed in EPA 537[45] (Supporting 293
Information Table 1). 294

Manual curation of detected chemical features yielded 58 295
distinct chromatographically resolved PFAS peaks, correspond- 296
ing to 38 unique molecular formulas. Recent efforts have been 297
made to identify novel contaminant species in fluorochemically 298
impacted water supplies by the US EPA and other 299
groups.[26,46−49] Cross-referencing literature lists of expected 300
monoisotopic masses and their hypothetical structures against 301
the molecular features observed in this study accounts for 302
roughly half of the unique features, with the remainder being 303
unidentified based on literature comparison. 304

The features were individually examined to determine novel 305
structures as described in the Methods section (Supporting 306
Information Table 2). For each mass-to-charge (m/z) 307
identified at the formula level or better, an extracted ion 308
chromatogram of the precursor mass was generated to 309
determine retention times (Supporting Information Figures 310
1−83). Composite MS/MS spectra consisting of multiple 311
averaged MS/MS scans at 10 and 25 CID settings were 312

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Table 1. Identified Perfluorinated Ether Acids

| ID	Conf	Formula	Theoretical [M]	ppm error	Isomers	CAS	Ref
1	1	$C_3HF_5O_3$	179.9846	1.68	1	674-13-5	29
2	1	$C_4HF_7O_3$	229.9814	2.18	2	13140-29-9, 377-73-1	29
3	1/2b	$C_4HF_7O_4$	245.9763	0.41	1	39492-88-1	29
4	2b	$C_5HF_9O_3$	279.9782	1.43	1	267239-61-2	29
5	3	$C_5HF_9O_4$	295.9731	2.71	1		
6	1	$C_5HF_9O_5$	311.968	0.96	1	39492-89-2	29
7	1	$C_6HF_{11}O_3$	329.975	1.22	1	13252-13-6	29
8	3	$C_6HF_{11}O_4$	345.9699	0.58	1		
9	3	$C_6HF_{11}O_5$	361.9648	0.83	1		
10	1	$C_6HF_{11}O_6$	377.9597	1.59	1		29
11	4	$C_8HF_{13}O_4$	407.9667	0.25	1		
12	3	$C_7HF_{13}O_5$	411.9616	1.46	1		
13	1	$C_7HF_{13}O_7$	443.9515	0.90	1		29
14	2b	$C_4HF_9O_4S$	315.94518	1.97	1	113507-82-7	29
15	3	$C_5HF_{11}O_4S$	365.94199	0.85	1		
16	1	$C_7HF_{13}O_5S$	443.93371	0.20	1	29311-67-9	29
17	3	$C_8HF_{15}O_5S$	493.93052	0.04	1		
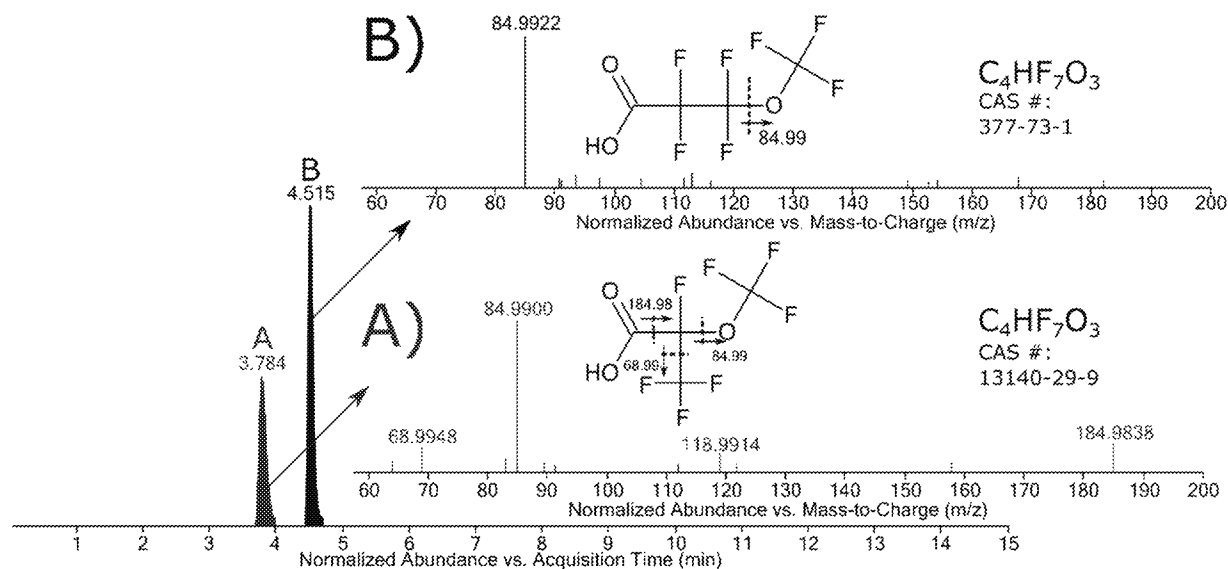
Figure 1. Determination of isomeric branching of perfluorinated ether compounds based on MS/MS fragmentation patterns. Compound 2A elutes at 3.8 min and has multiple major mass fragments. Compound 2B elutes later at 4.5 min and fragments only at the terminal ether linkage.

313 generated for individual isomers of each precursor, where
314 possible (Supporting Information Figures 1–83).
315 **Group A: Perfluorinated Ether Acids.** A frequently
316 observed PFAS class is that of fluoroether acids (here Group
317 A), which exist with a general formula
318 $CF_3(CF_2)_n(CF_2O)_m(R_1)$, where the headgroup, R_1, is an
319 acidic moiety such as $-COOH$ or $-SO_3H$.[29,36] These were
320 expected to be the primary contaminants identified in the
321 survey, given that nearby production includes perfluorinated
322 polyvinyl ethers, empirical formula $(CF_2)_n(CF_2O)_m$. The
323 production of the ammonium salt of 2,3,3,3-tetrafluoro-2-
324 (heptafluoropropoxy) propanoic acid,[21] where $n = 3$, $m = 1$, R1
325 = COOH is shown in the above scheme, and "Nafion", a
326 copolymer of tetrafluoroethylene and perfluoro(4-methyl-3,6-
327 dioxaoct-7-ene)sulfonic acid, a monomer where $n = 3$, $m = 2$,
328 R_1 = SO_3H is shown as above.[50] Numerous molecules in this
329 series were found during this sampling event (Table 1).
330 Compound 2 was identified previously in historical sampling
331 in the river basin;[29] however, the UPLC separation resolved

332 two isomeric structures with different MS/MS fragmentation
333 patterns (Figure 1), which was not observed for other Group A
334 species. The more abundant isomer of compound 2 (2B) is
335 available as a standard, and its MS/MS fragmentation pattern
336 and retention were confirmed to match the tentative
337 assignment. The additional isomer is currently not commer-
338 cially available and was assigned on the basis of MS/MS
339 fragmentation and the shift in reverse phase retention time,
340 indicative of increased branching (Schymanski Level 2b).[51]
341 The primary fragments for perfluorinated ether carboxylic
342 acids are typically decarboxylation (neutral loss of 43.9898 Da)
343 and production of the terminal ether fragment (here CF_3O^-,
344 84.99067 Da). Sulfonated acids produce a sulfonate (SO_3^-,
345 79.9568 Da) fragment instead of a decarboxylation, but the
346 primary structural fragment remains the terminal ether. These
347 patterns were observed across all Group A compounds, but the
348 lack of significant internal fragmentation makes it difficult to
349 assign complete linkage isomerization of longer perfluorinated
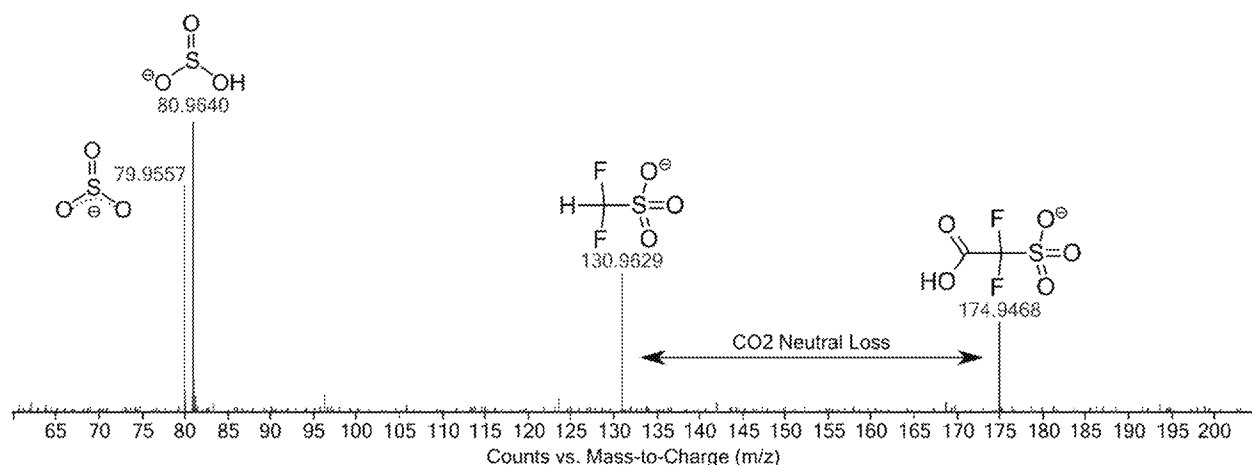
Figure 2. Annotated MS/MS spectrum of precursor m/z 174.9496 (compound 18), assigned the molecular formula $C_2H_2F_2O_5S$.

Table 2. Identified Perfluorinated Ether Acids with Multiple Acidic Sites

ID	Conf	Formula	Theoretical [M]	ppm error	Isomers	CAS	Ref
18	2b	$C_2H_2F_2O_5S$	175.9591	0.57	1	422-67-3	55
19	2b/3	$C_3H_2F_8O_6S$	341.9444	2.35	2	790642-73-8	55
20	3	$C_6H_2F_{10}O_5$	343.9743	2.33	1	919005-07-5	56
21	4	$C_7H_3F_{11}O_7S$	439.9424	0.68	2		
22	2b/3	$C_7H_2F_{12}O_6S$	441.938	0.23	2	852157-01-8	57
23	2b/3	$C_8H_2F_{14}O_7S$	507.9298	1.18	2	1235024-21-1	57
24	2b/3	$C_7H_3F_{13}O_8S_2$	525.9062	0.19	2	916340-65-3	58

350 ethers based on MS/MS alone, and many compounds remain
351 level 3 identifications.
352 The initial patent description of the polyvinyl ether process
353 refers to the creation of multiple competing side reactions and
354 low molecular weight polyfluoro-ether byproducts,[52,53] which
355 have been used as reactants and starting materials in more
356 complicated copolymer production schemes.[54] Without further
357 information about manufacturing processes, it is not
358 immediately clear which reactions and waste streams yield
359 the perfluoroether compounds detected and whether they are
360 primary or secondary waste products. Further, broader
361 nontargeted efforts would be necessary to determine whether
362 they are contained in finished products directly impacting
363 consumers or only in the nearby environment; however, the
364 spatial and temporal correlation of detected species is highly
365 suggestive of the point source (i.e., manufacturing outfall).
366 **Group B: Multi-Headed Perfluoroether Species.**
367 Perfluoroether species previously described can be thought
368 of in a more general sense as compounds with the form R_1-
369 $(CF_2)_n(CF_2O)_m$-R_2, where R_1 and R_2 are both variable head
370 groups. While R_1 is CF_3 for the Group A perfluorinated acids
371 previously discussed, some compounds were observed with
372 multiple acidic groups (R_1, R_2 = $-COOH$ or $-SO_3H$). This
373 group of compounds was identified in both -1 and -2 charge
374 states and had initial predicted formulas suggesting an
375 overabundance of oxygens which could only be structurally
376 supported by multiple acidic sites on the perfluorinated
377 backbone. Further, diagnostic ions for CO_2 and/or SO_3 were
378 observed (Figure 2). We grouped these species as Group B,
379 multiply sulfonated and/or carboxylated perfluorinated ether
380 acids (Table 2).
381 While these species have not been previously reported in
382 environmental media, to the best of our knowledge, the
383 literature of fluorochemical manufacturers proposes a few

384 possible structures in this group, primarily as side products in
385 fluoropolymer production and degradation.[55-57] The forma-
386 tion of short chain fluorosulfonyl ($-SO_2F$) and acyl fluoride
387 ($-COF$) fluoroethers formed as byproducts or intermediate
388 steps in fluoropolymer synthesis is the likely source of
389 precursor compounds that degrade into the observed
390 compounds.[52] Aqueous hydrolysis of the acid fluoride
391 intermediates during production or water treatment yields
392 the familiar carboxylic acid ($-COOH$) and sulfonic acid
393 moieties ($-SO_3H$), while more basic conditions (e.g., KOH,
394 Na_2CO_3, etc.) yield further hydrolysis products (Scheme 1). s1
395 These full and partial hydrolysis reactions are well-known steps
396 in fluoropolymer production and in the abatement of
397 fluoroether salts.[59-61]

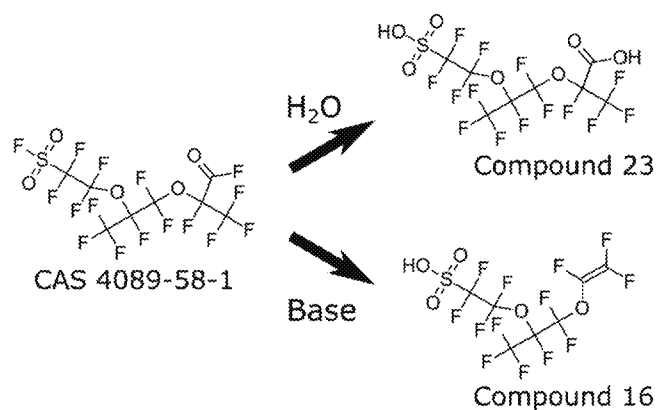**Scheme 1. Transformation of a Fluorosulfonyl/Acyl Fluoride Fluoroether to Sulfonate End Products by Hydroloysis**

Table 3. Identified Polyfluorinated Ether Acids with a Single Hydrogen Substitution

ID	Conf	Formula	Theoretical [M]	ppm error	Isomers	CAS	Ref
25	2b/3	$C_3H_2F_6O_4S$	247.9578	1.62	3	905363-45-3	58
26	1	$C_4H_2F_8O_4S$	297.9546	1.35	1	905363-44-2	58
27	2b/3	$C_5H_2F_{10}O_4S$	347.9514	1.15	3	935443-55-3	58
28	3	$C_6H_2F_{12}O_4S$	397.9482	1.51	2		
29	3	$C_8H_2F_{14}O_4S$	459.9450	0.00	3		
30	2b/3	$C_5H_2F_8O_3$	261.9876	1.15	3	919005-00-8, 919005-13-3	56
31	2b/3	$C_6H_2F_{10}O_3$	311.9844	1.61	3	919005-19-9	56
32	3	$C_7H_2F_{12}O_3$	361.9812	3.05	1	919005-26-8	56
33	3	$C_6H_2F_{12}O_5S$	413.9431	0.00	2		
34	1	$C_7H_2F_{14}O_5S$	463.9399	1.30	2	749836-20-2	29
35	4	$C_9H_2F_{16}O_6S$	541.9317	0.00	1		
36	1	$C_8H_2F_{14}O_4$	427.9730	2.11	1	773804-62-9	
37	4	$C_8H_2F_{14}O_7$	475.9577	4.42	2		

Group C: Single-H Containing Polyfluorinated Acids.

Another subset of fluorinated compounds, Group C, was identified with molecular formulas containing a single F → H substitution compared to that in the Group A perfluorinated ether compounds (Table 3). For example, compound 14 ($C_4HO_4SF_9$) and compound 26 ($C_4H_2O_4SF_8$) differ by the substitution of a hydrogen for a fluorine. These compounds are highly isomerized, and MS/MS fragmentation is frequently insufficient to unequivocally assign the structure; the structures presented in the Supporting Information (Table S-2) are representative of a single observed isomer.

The production of polyfluorosulfonic acid ethers of the form $R-OCFH-CF_2-SO_3$ was described in early patent filings,[58] and their use as fluoropolymer surfactants was mentioned in a follow up.[62] The use and production of these chemicals as part of Nafion manufacturing is assumed to be responsible for at least some of the observed species. In contrast to the previously mentioned literature structures, MS/MS fragmentation of some polyether sulfonate isomers indicates that the single hydrogen substitution occurs on the tail ether rather than adjacent to the head; for example, the fragment C_2HF_4O- (116.9969 Da) from one isomer of compound 30 (Supporting Information Figure 64) is derived from a two carbon polyfluorinated ether unit (Supporting Information Table S-4). These species are assumed to be products of the fluorosulfonyl and/or acyl fluoride species which produced Group B but further decomposed. The complete loss of a headgroup (e.g., decarboxylation) during production, waste treatment, or naturally in surface water would yield a compound with a single hydrogen in that site (Scheme 2). Thermally decarboxylated perfluoroether species are noted as potentially undesirable byproducts of fluoropolymer production in the patent literature of fluoropolymer production,[60] and partially decarboxylated species are noted as possible products from multiply acidic species such as Group B chemicals.[63]

Presence and Prevalence of Novel Species. The lack of authentic standards limits the potential for quantitative measurements of novel compounds; relative abundance and semiquantitative estimates of emerging compounds are all that are available. However, this information remains useful for targeting individual chemicals for follow-up study and for monitoring environmental changes in response to systemic actions. Our examination of the May 2017 downstream sample could assign 35 of the top 50 most abundant features, corresponding to ~90% of the total ion current in the sample (Figure 3). While ion abundances are sensitive to many aspects of the sample matrix and molecules, the structural similarity of the fluoroethers would suggest some similarity in instrument response, and that species can be roughly compared. At least one species of each of the three major chemical groups outlined (groups A, B, and C) exhibited a higher ion abundance than the HFPO-DA species with a targeted health goal. Further, of the targeted compounds detailed in EPA 537,[45] only perfluoropentanoic acid (PFPeA) is present in the top 50; thus, the fluorochemical load in the system would be greatly underestimated by traditional targeted approaches. Additionally, including the compounds previously detailed in studies of the Cape Fear River[29,30] accounts for only ~50% of the total ion intensity in the initial sample, suggesting that even single-time-point nontargeted survey can fail to sufficiently capture the chemical complexity of a location.

An advantage of nontargeted analysis is the historical data banking of samples and the ability to perform relative quantification across disparate sample sets. The comparison of chemical species abundance over the time period from May 2017 to February 2018 revealed several distinct abundance patterns from simple visual inspection (Figure 4). Many fluoroether species exhibited exponential decline in concentration (Figure 4, left) while others showed lagging or even temporarily increased abundances (Figure 4, center and right).

Matrices of the weekly relative ion abundance of each of the identified chemical features over the eight sampling points were clustered for similarity using Ward's clustering criterion implemented in R *hclust*;[38] *p*-value calculations for clusters were performed using multiscale bootstrap resampling implemented in the *pvclust* package. Clustering of the time-trend data yielded six distinct chemical clusters (Figure 5, Supporting Information Figures 84−89).

Scheme 2. Transformation by Decarboxylation of Sulfonated Carboxylic Acid Perfluoroether to Yield the Polyfluorosulfonic Acid
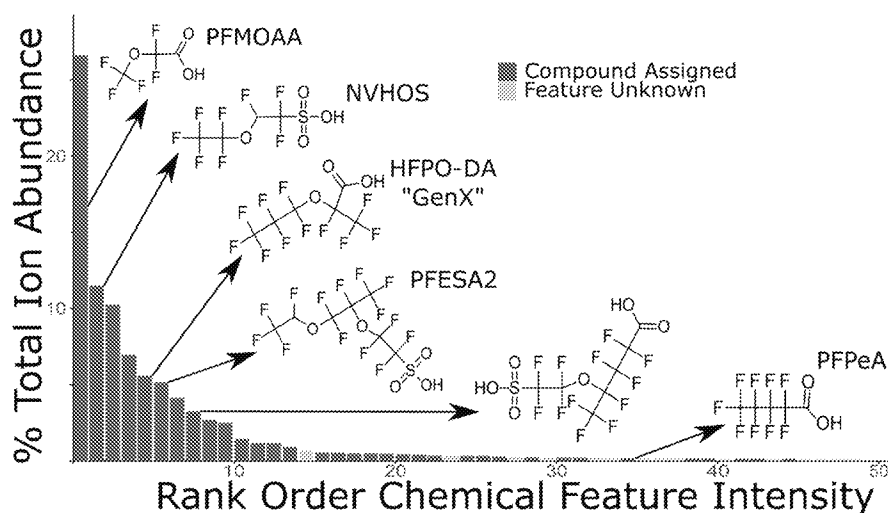

Compound 19 ➡ Compound 26
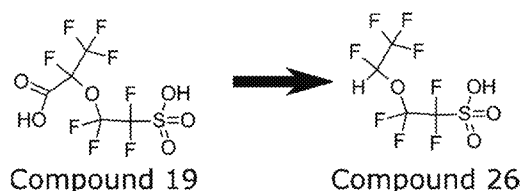
Figure 3. Total ion abundance as a percentage of the sum of all chemical features for compounds identified in a May 2017 sample of the Cape Fear River downstream of a fluorochemical manufacturer. Selected highly abundant compounds confirmed by standards obtained from and labeled by Chemours include: compounds 1 (PFMOAA), 26 (NVHOS), 7 (HFPO-DA), 34 (PFESA2), 22 (PFESA4), and perfluoropentanoic acid (PFPeA).
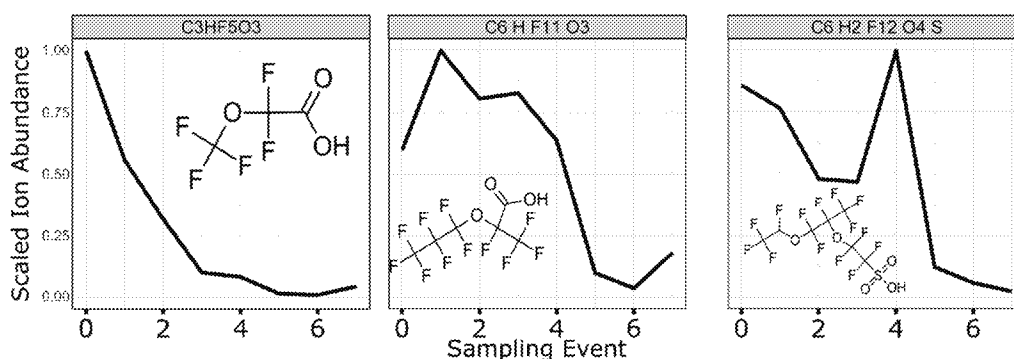

Figure 4. Scaled ion abundance of three chemical features confirmed by standard and detected in the Cape Fear River over a time course sample. Time points one to six were taken weekly beginning June 19, 2017 with time points zero and seven being collected in May 2017 and February 2018, respectively.

476 These clusters support the hypothesis that multiple
477 production processes generate distinct groups of the chemicals
478 observed in the Cape Fear River, as the compound abundances
479 are correlated over time and are chemically similar based on
480 naive classification. For example, the cluster at branch 16
481 (Figure 5) contains the H-substituted sulfonic acid ethers from
482 Group C. These compounds abruptly and almost uniformly
483 vanish following the initial sampling event (Supporting
484 Information Figure 86), while the clusters at branches 13
485 and 18 contain the perfluoroether carboxylic acids and their
486 associated H substituted versions; both classes exponentially
487 and smoothly decrease from the early sampling events
488 (Supporting Information Figures 84,87). Other clusters
489 contain more chemical variation, as well as more complex
490 time-trend patterns, that may be reflective of species with
491 multiple sources or processes involving multiple PFAS classes.
492 It is worth noting that the chemical assignment provided to
493 each feature is not required for clustering. Application of the
494 clustering approach reveals chemical feature relationships that
495 could be used to target unidentified species for follow-up
496 analysis based on their close association with compounds of
497 interest (e.g., HFPO-DA), and occurs before the assignment of
498 formulas or structures.
499 **Environmental Impact.** The phase-out of PFOA/PFOS
500 and longer-chain PFAS materials has led to an increased

501 prevalence of replacement chemicals. The chemical groups
502 detailed here are derived from processes and procedures that
503 are over 20 years old within the fluorochemical production
504 industry but are only now becoming apparent due to increased
505 scrutiny of environmental media and the proliferation of
506 nontargeted methodologies. Fluorinated ethers such as those
507 described here have been reported in the Cape Fear River
508 previously.[29,30] Similar structures have been reported in
509 numerous locations worldwide, indicating that emerging
510 PFAS are every bit as ubiquitous as their PFOA/PFOS
511 antecedents.[64,65] Physiochemical characteristics of similar
512 perfluorinated ether compounds suggest that the fluoroether
513 substances are of reduced health impact compared to similarly
514 sized perfluorinated acids, but retain some degree of health
515 risk.[66] Further, longer-chain perfluorinated ethers are not
516 currently regulated similarly to long chain perfluorinated acids
517 and exhibit similar bioaccumulation potential, as evidenced by
518 the preliminary reporting in human blood in spite of their
519 relative low abundance in surface water.[67] Efforts to determine
520 reasonable health levels for these emerging compounds are
521 ongoing. During the preparation of this manuscript, the state of
522 North Carolina set a drinking water health goal for a single
523 perfluorinated ether acid (PFECA) compound, HFPO-DA, at
524 140 ng/L, based on their assessment of human health risk from
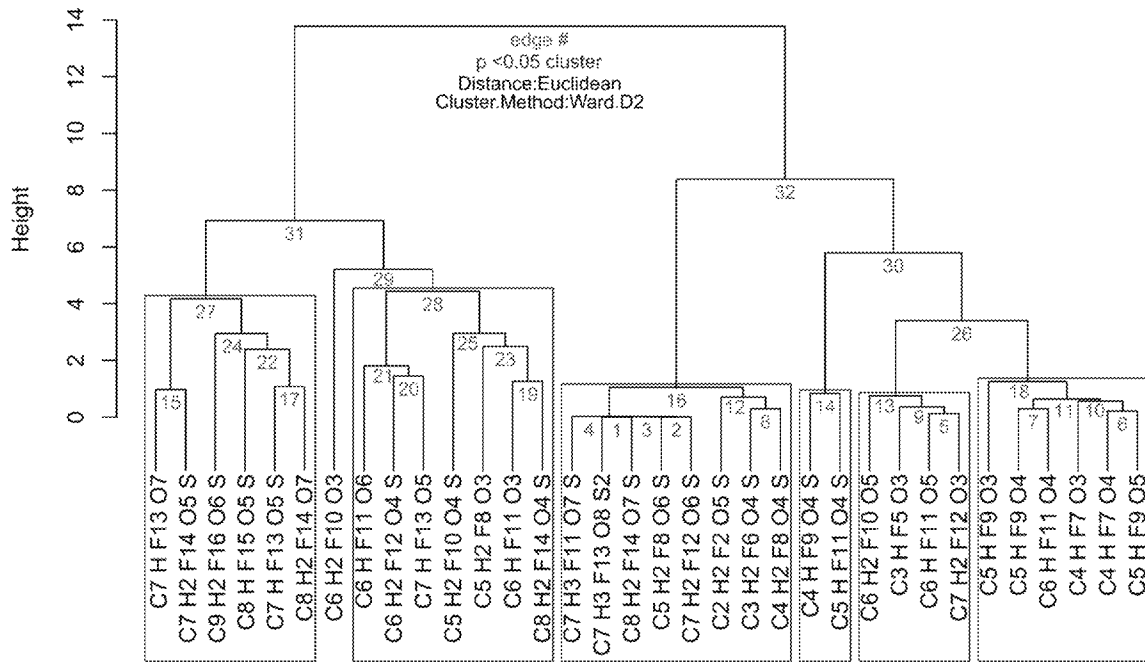525 available data submitted in the European Union.[31,68,69]

G

Figure 5. Clustering of identified chemical features from scaled ion-abundance of features detected in the Cape Fear River in eight sampling events between May 2017 and February 2018.

526 Sulfonated ether substances in this measurement series are
527 primarily associated with sulfonated fluoropolymer production,
528 specifically the Nafion polymer, and are of lesser widespread
529 concern due to the limited manufacture of such polymers and
530 their primary application being energy-related technologies,
531 such as fuel cells.[50] Nevertheless, for locations that are exposed
532 to such manufacturing waste streams, sulfonated fluorinated
533 acids have shown higher accumulation potential and
534 toxicological effects than equivalent carboxylic acids.[19] Further,
535 other sulfonated ether acids, such as F-53B, have shown
536 equivalent bioaccumulation potential as the regulated per-
537 fluorinated sulfonic acid PFOS.[70,71] Other sulfonated fluo-
538 roether species have begun to be detected by other groups, but
539 they exhibit different structural elements and likely have
540 different origins that need to be examined independently.[64,72]
541 The continued appearance of new PFAS species necessitates
542 an increase in the generation of toxicological profiling and
543 monitoring efforts to assess human risk from exposure to these
544 novel chemicals.
545 With regards to human exposure, it has been demonstrated
546 that perfluoroether compounds are poorly cleared by water
547 treatment processes, including activated carbon, and the most
548 effective method of removal is through source control.[30,73]
549 Long-term monitoring of impacted watersheds remains an
550 important tool for both detecting new chemical species over
551 multiyear time scales and for determining shorter-term changes
552 in local chemical load. An application of this approach to other
553 watersheds has been effective for other chemical classes, such
554 as surfactants.[23,47] During the course of this study, our
555 monitoring activities resulted in the cessation of discharge from
556 the vinyl ether production at the source manufacturing
557 location, which resulted in a concomitant decrease in PFECAs
558 hypothesized to derive from that process, but not in the
559 immediate reduction of the perfluorinated ether sulfonic acid
560 (PFESAs) species since they derive from a different source
561 than the PFECAs.[33] Targeted methods and single time-point
562 nontargeted surveys can easily miss transient chemicals or

563 underestimate chemical loads for exposure assessment; some
564 species spiked as much as 5-fold during the sampling period
565 (cluster branch 27, Supporting Information Figure 88), even as
566 other compounds were declining. The proactive application of
567 a nontargeted screening approach is capable of identifying and
568 monitoring chemicals present in the water, even prior to
569 complete structural elucidation or toxicological analysis and
570 provides a useful tool toward those ends.

■ ASSOCIATED CONTENT   571

S Supporting Information   572

The Supporting Information is available free of charge on the   573
ACS Publications website at DOI: 10.1021/acs.est.8b06017.   574

All detected chemical features, assigned chemical   575
species, software settings for molecular feature extrac-   576
tion, common MS/MS fragments of PFAS compounds,   577
chromatographic traces for molecular ions of chemical   578
features, composite MS/MS fragmentation spectra of   579
chemical features, scaled abundance time trends for   580
chemical features, statistical validation of chemical   581
feature clustering, sampling map (PDF)   582
Cape Fear upstream and downstream data (XLSX)   583

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The manuscript was written through contributions of all   591
authors. All authors have given approval to the final version of   592
the manuscript.   593

Notes   594

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